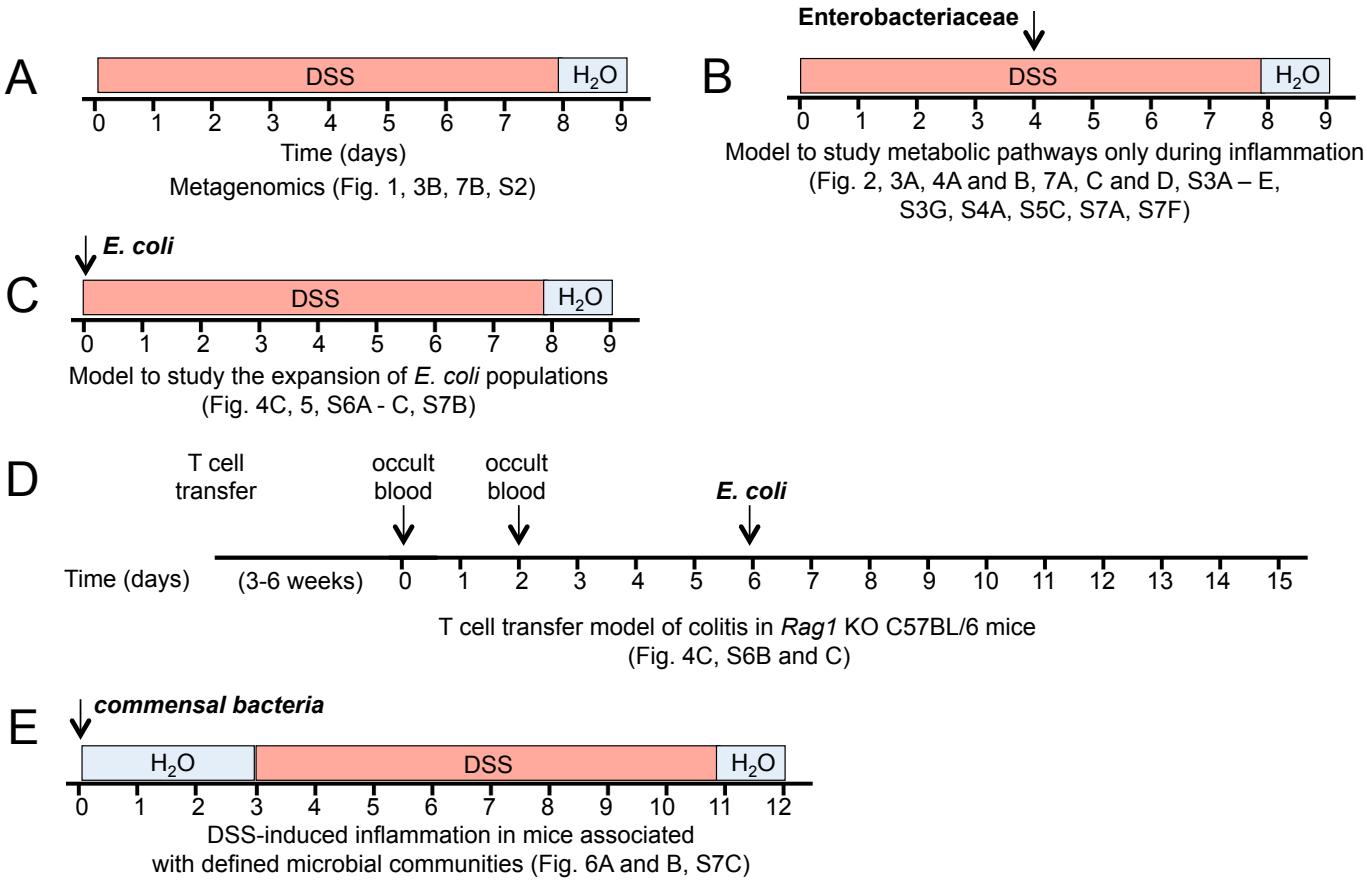

F

e^- e^- e^- e^-

Electron donor	Respiratory Dehydrogenase	Quinone pool	Terminal Reductase / Oxidase	Electron acceptor
NADH	Ndh (NDH-1)	Ubiquinone Menaquinone Demethyl-menaquinone	CyoABCD CydAB CyxAB	Oxygen
	Nuo (NDH-2)			
Formate	FdnGHI (FDN)		NapABCGH NarGHI NarZYV	Nitrate
	FdoGHI (FDO)			
Hydrogen	HyaABC			
	HybCOAB			
Succinate	SdhCDAB		DmsABC YnfFGH TorACD TorZY YedYZ	*S*-oxides and *N*-oxides
Glycerol-3-Phosphate	GlpABC			
	GlpD			
Lactate	Dld			
	LldD			
Pyruvate	PoxB		NrfABCD	Nitrite
Malate	Mqo		FrdABCD	Fumarate
Glucose	Gcd			

Figure S1. Related to Fig. 1-7: (A - E) Schematic representations of colitis models used in this study. For details, please see text. **(F)** Electron donors, electron acceptors, and enzymes involved in the respiratory chains in *E. coli*. Electron donors are oxidized by membrane-bound dehydrogenases and the electrons liberated by this reaction are transferred to the quinone pool. Electrons are then shuttled to terminal reductases and oxidases, which reduce the terminal electron acceptor. Proton motive force is generated either by active proton translocation (proton pumps) or by scalar chemistry. Certain combinations of electron donating and accepting reactions do not occur under physiological conditions due to incompatibility of the respective reduction potentials. Molybdopterin cofactor-dependent enzymes are shaded in light blue.

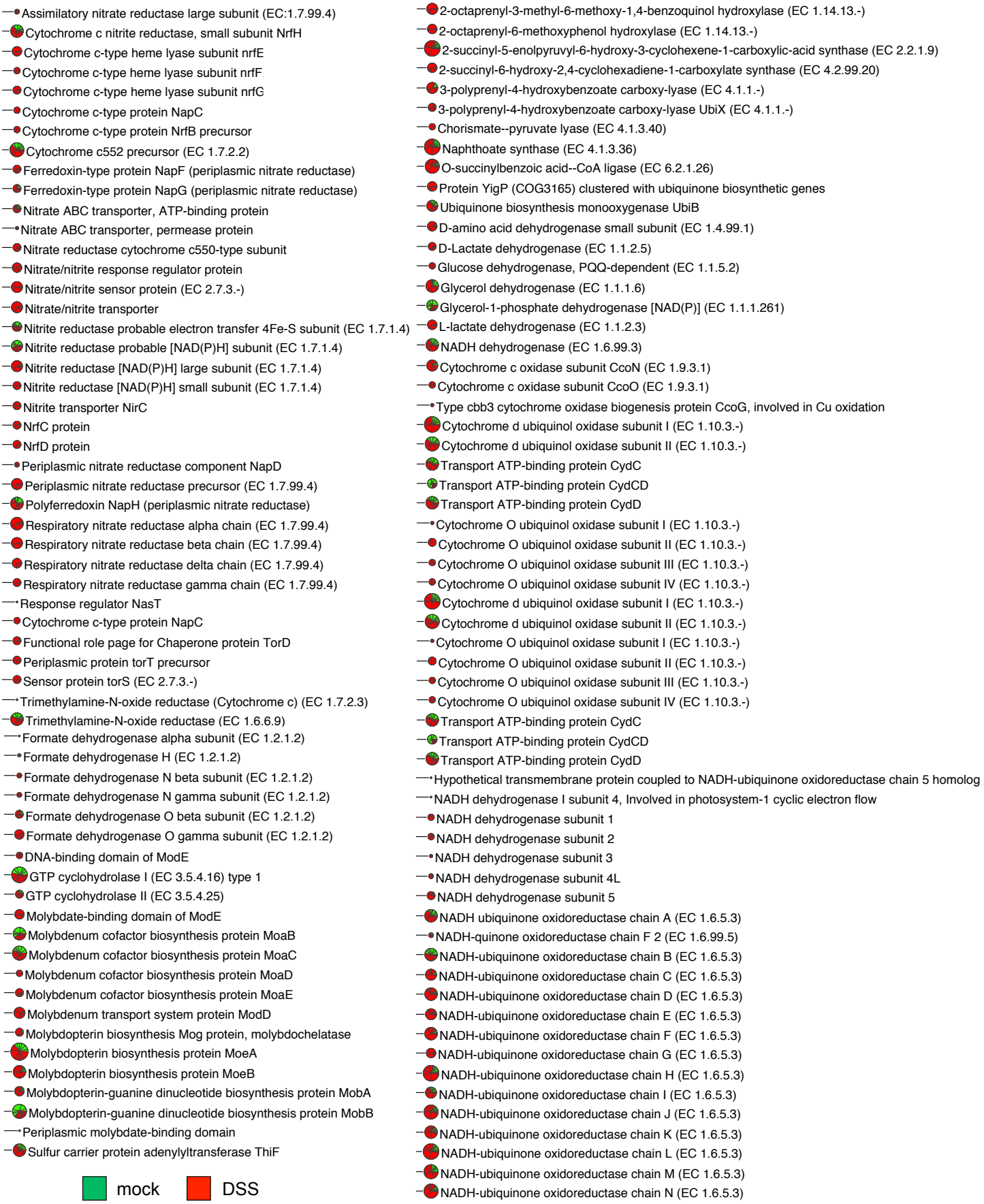

Figure S2. Related to Fig. 1: Shotgun metagenomic sequencing of the gut microbiome. DNA sequences from the metagenomic analysis shown in Fig. 1 were assigned to functional categories using the SEED algorithm (MEGAN6). Green and red portions of the circle chart represent mock-treated and DSS-treated animals, respectively.

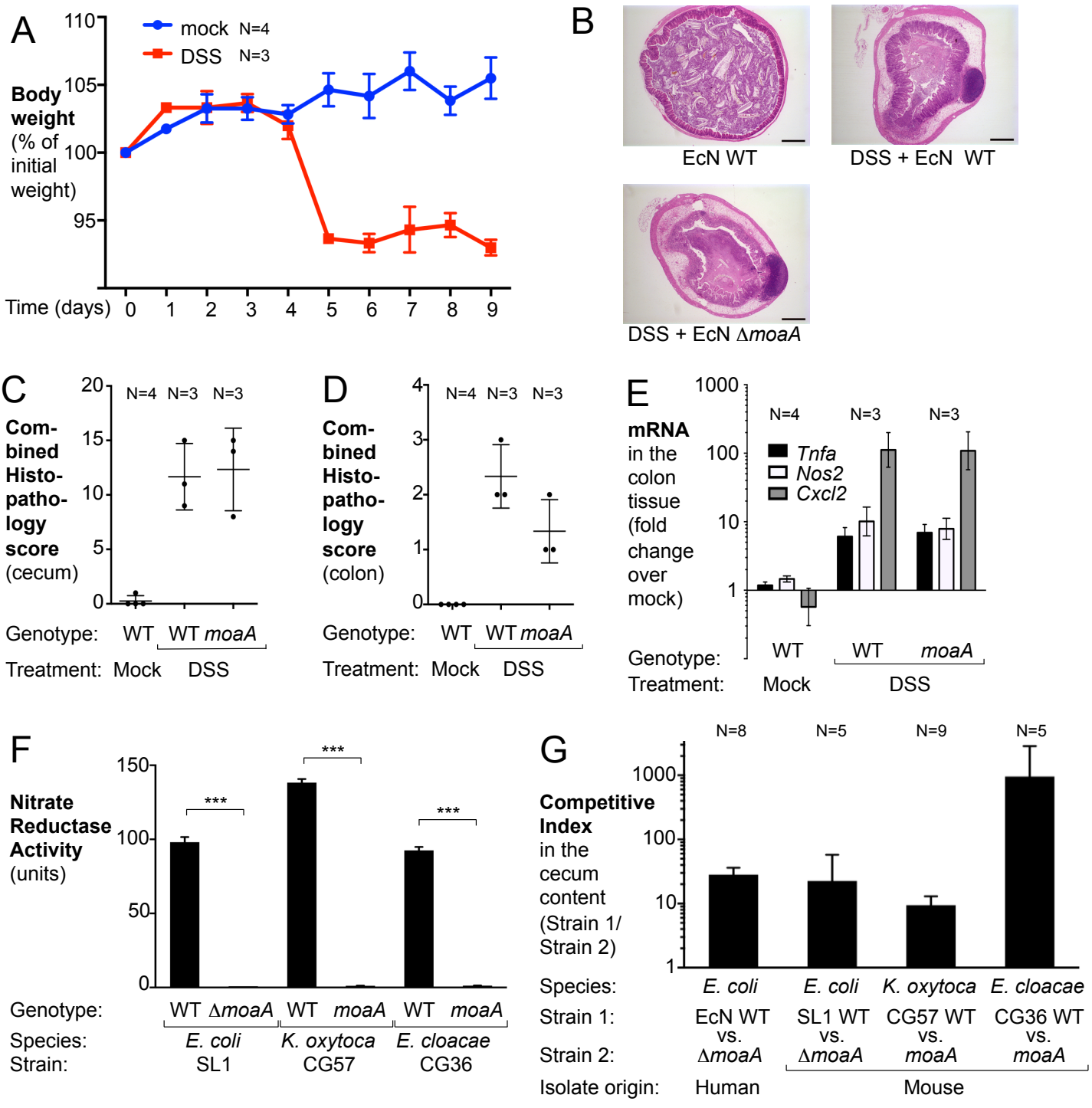

Figure S3. Related to Fig. 2: Contribution of molybdopterin cofactor-dependent enzymes to gut colonization in the dextran sulfate sodium (DSS) colitis model. (A) Change in body weight of C57BL/6 mice that received 3 % DSS in the drinking water (red line; N = 3) or were mock treated (blue line; N = 4), and intragastrically inoculated with *E. coli* Nissle 1917 (EcN) wild-type on day 4. **(B-E)** Inflammation analysis of mice also shown in Fig. 2A – C and S3A. Mock or DSS - treated (3 %) animals were orally inoculated with the EcN wild-type strain or the molybdopterin cofactor biosynthesis-deficient *moaA* mutant. **(B)** Representative images of hematoxylin and eosin-stained colonic sections. Scale bar, 500 μm. **(C And D)** Combined histopathology score of lesions in the cecum **(C)** and colon **(D)**. The centerlines represent means ± standard deviation. **(E)** mRNA levels of pro-inflammatory markers were determined by RT-qPCR. **(F)** Nitrate reductase activity in mouse commensal Enterobacteriaceae isolates SL1, CG57, and CG36. Bacterial strains were subcultured microaerobically for 3 h in the presence of 40 mM sodium nitrate and nitrate reductase activity determined using a modified Griess assay. The experiment was performed in 3 independent biological replicates. **(G)** DSS-treated (3 %) C57BL/6 mice were inoculated with an equal mixture of the indicated wild-type strain and the respective *moaA* mutant (animals also shown in Fig. 2D). Five days after inoculation, the competitive index in the cecum content was determined. Bars represent geometric means ± standard error. The number of mice per group (N) is indicated in each panel. ***, $P < 0.001$

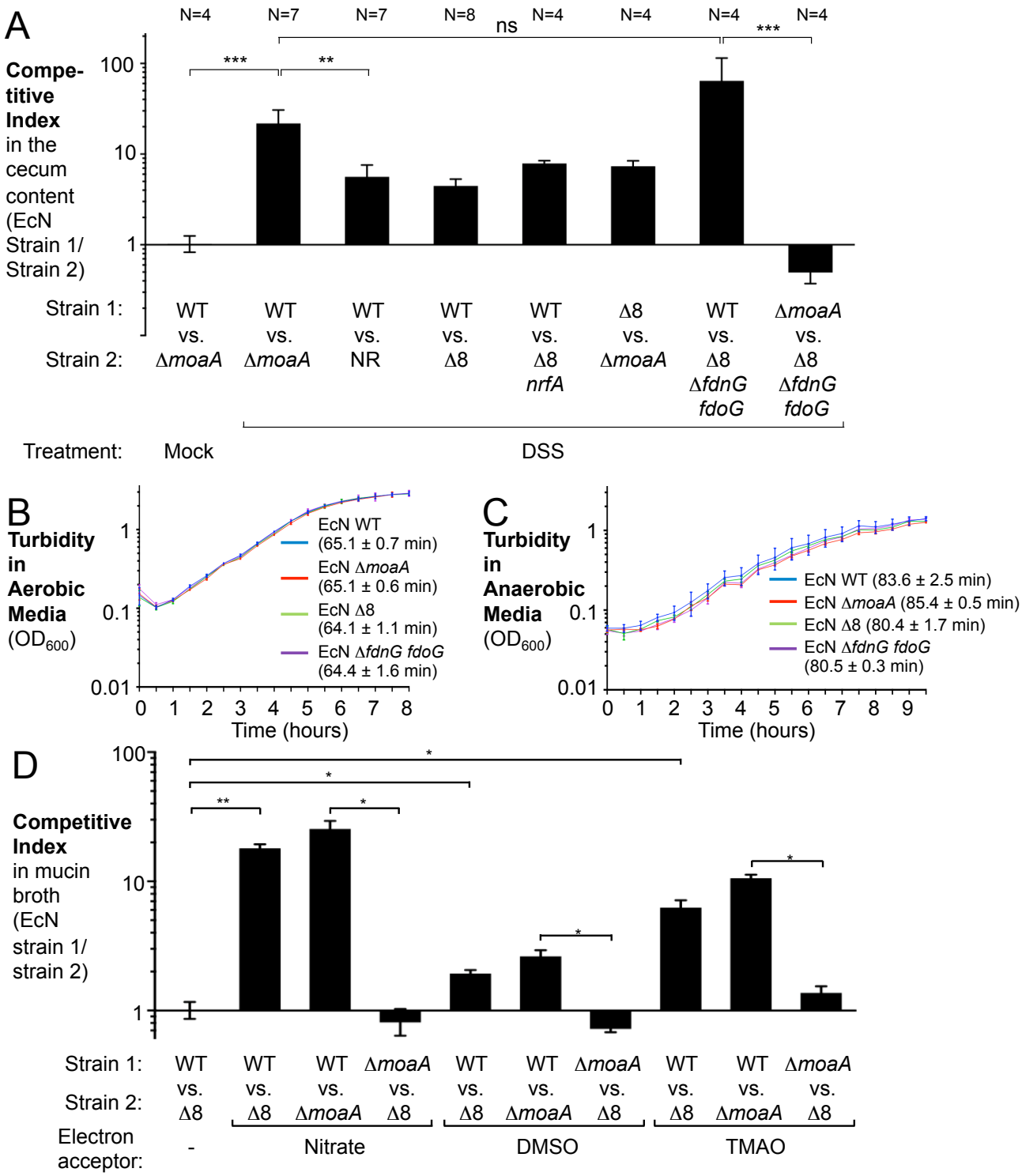

Figure S4. Related to Fig. 3: Molybdopterin cofactor-dependent pathways contribute to fitness of *E. coli* Nissle 1917 (EcN) in the inflamed cecum. (A) DSS- (3%) and mock-treated animals were inoculated with the indicated strains. Five days after inoculation, the competitive index was determined in the cecum content (animals also shown in Fig. 3A). Bars represent geometric means ± standard error. WT, wild-type strain, NR, *narG napA narZ* mutant; Δ8, *narG napA narZ torA torZ yedY dmsA ynfF* mutant. The number of mice (N) is indicated above each bar. **(B)** Aerobic and **(C)** anaerobic growth of the indicated EcN strains in M9 minimal media supplemented with glucose. Data points represent averages ± standard deviation from three independent biological replicates. In parentheses are the generation times ± standard deviation. **(D)** Mucin broth, supplemented with the indicated electron acceptors at a final concentration of 40 mM, was inoculated with an equal mixture of the EcN WT and the isogenic Δ8 mutant, the WT and an isogenic Δ*moaA* mutant, or the Δ*moaA* and the Δ8 mutant, respectively. Cultures were incubated anaerobically for 16 h and the ratio of the two strains (competitive index) determined. Bars represent geometric means ± standard error from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant.

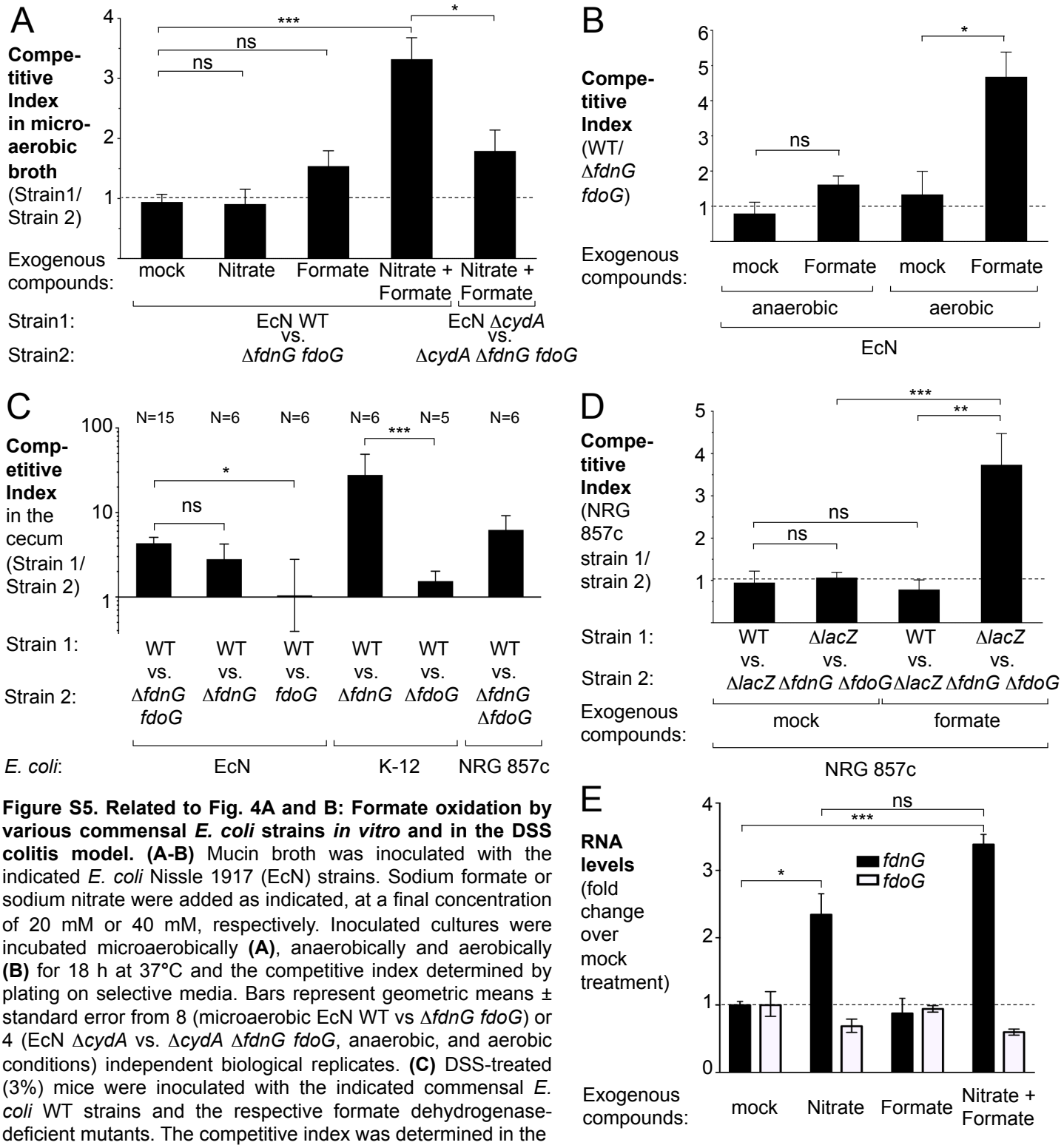

Figure S5. Related to Fig. 4A and B: Formate oxidation by various commensal *E. coli* strains *in vitro* and in the DSS colitis model. (A-B) Mucin broth was inoculated with the indicated *E. coli* Nissle 1917 (EcN) strains. Sodium formate or sodium nitrate were added as indicated, at a final concentration of 20 mM or 40 mM, respectively. Inoculated cultures were incubated microaerobically **(A)**, anaerobically and aerobically **(B)** for 18 h at 37°C and the competitive index determined by plating on selective media. Bars represent geometric means ± standard error from 8 (microaerobic EcN WT vs Δ*fdnG fdoG*) or 4 (EcN Δ*cydA* vs. Δ*cydA* Δ*fdnG fdoG*, anaerobic, and aerobic conditions) independent biological replicates. **(C)** DSS-treated (3%) mice were inoculated with the indicated commensal *E. coli* WT strains and the respective formate dehydrogenase-deficient mutants. The competitive index was determined in the cecum content (animals also shown in Fig. 4A). The number of mice per group (N) is indicated. **(D)** Mucin broth, supplemented with formate as indicated, was inoculated with an equal mixture of the Adherent Invasive *E. coli* isolate NRG 857c and an isogenic Δ*lacZ* mutant or the Δ*lacZ* mutant and an isogenic Δ*fdnG* Δ*fdoG* mutant. The competitive index after 16 h of anaerobic growth at 37 °C was determined by plating on chromogenic agar plates. Bars represent geometric means ± standard error from four independent biological replicates. **(E)** RNA levels of fdnG and fdoG were determined by RT-qPCR in WT EcN. Mucin broth was inoculated with EcN for 3 hours microaerobically at 37 °C with sodium formate or sodium nitrate added, as indicated. Experiment was performed in triplicate and a paired *t*-test was used to calculate statistical significance. Bars represent geometric means ± standard error. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant.

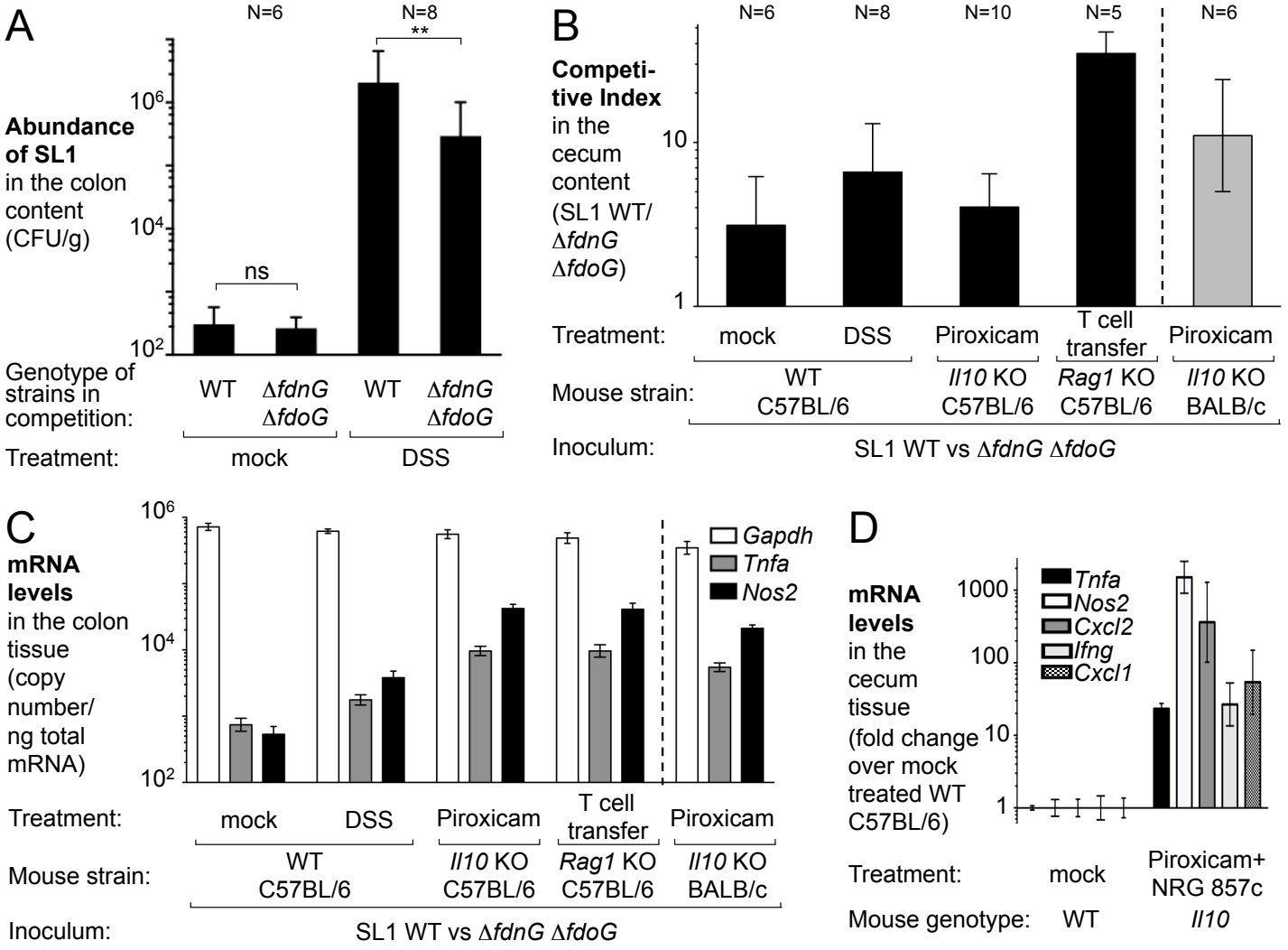

Figure S6. Related to Fig. 4: Formate dehydrogenases contribute to fitness of commensal *E. coli* SL1 and the AIEC NRG857c in multiple animal models of colitis. (A) Mice were orogastrically inoculated with a mixture of the SL1 wild-type strain (WT) and the Δ*fdnG* Δ*fdoG* mutant and treated with DSS (2 %) for 9 days or mock-treated (animals also shown in Fig. 4C). Individual populations in the colon from the competitive colonization experiment shown in Fig. 4C. Samples were compared using paired *t*-test. **(B)** The cecal competitive index of the commensal *E. coli* WT strain SL1 and the isogenic Δ*fdnG* Δ*fdoG* mutant in the DSS colitis model (see above), in piroxicam-treated *Il10*-deficient mice, and in a T-cell transfer model of colitis. For details, see Fig. 4 C. **(C)** mRNA levels of GAPDH (encoded by *Gapdh*; white bars) and the pro-inflammatory markers TNF-a (encoded by *Tnfa*; gray bars) and inducible nitric oxide synthase (iNOS, encoded by *Nos2*; black bars) in the colon tissue from mice described in Fig. 4C and Fig. S6A-B, as determined by RT-qPCR. **(D-E)** Three male *Il10*-deficient mice in the C57BL/6 background were treated with piroxicam and orally inoculated with an equal mixture of NRG 857c Δ*lacZ* (WT) and the Δ*fdnG* Δ*fdoG* strain. Cecal mRNA levels **(D)** of the pro-inflammatory markers TNF-α, iNOS, Cxcl2, IFNγ, and Cxcl1 (KC) encoded by *Tnfa* (black bars), *Nos2* (white bars), *Cxcl2* (medium gray bars), *Ifng* (light gray bars) and *Cxcl1* (dark gray bars) were determined by RT-qPCR. Also, the abundance of the indicated strains in the cecum content **(E)** were determined 7 days after inoculation. A paired *t*-test was used to test for significance. Bars represent geometric means ± standard error. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant. The number of mice per group (N) is indicated.

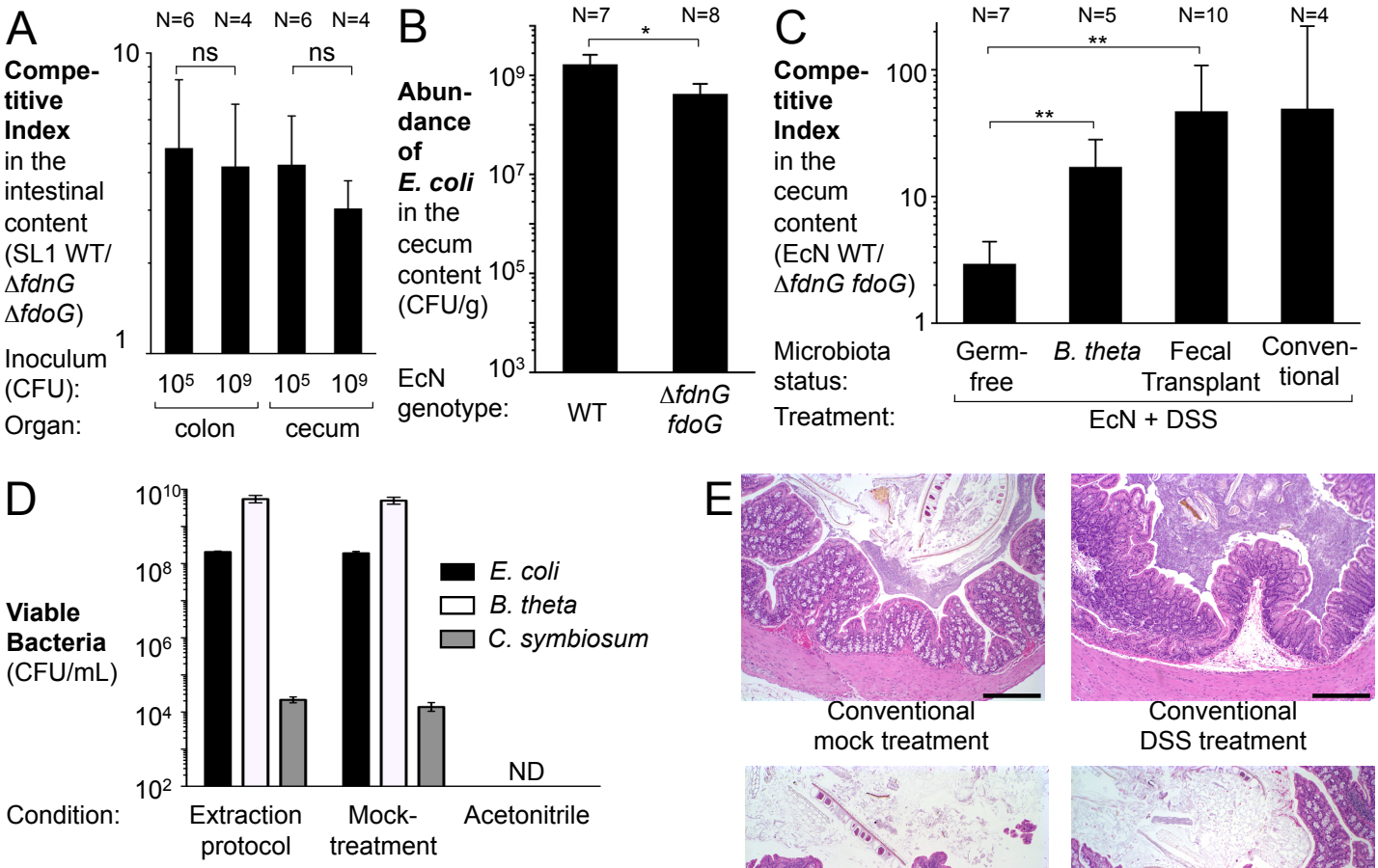

Figure S7. Related to Fig. 4-7: (A) DSS-treated (3 %) C57BL/6 mice were intragastrically inoculated with 10^9 or 10^5 CFU of *E. coli* SL1 wild-type (WT) and Δ*fdnG* Δ*fdoG* on day 4. The competitive index in colon and cecum content was determined 5 days later. **(B)** Groups of SPF mice were pre-colonized with the Nissle 1917 (EcN) WT or the Δ*fdnG fdoG* mutant. Inflammation was induced for 9 days by administration of 3 % DSS (animals shown in Fig. 5E). The bacterial load in the cecal content was determined by plating on selective media. **(C)** Cecal competitive index from animals also shown in Fig. 6A and B. Germ-free and conventional Swiss Webster mice received 3 % DSS for 9 days. Germ-free mice were pre-colonized for three days with *E. coli* alone, *E. coli* and *B. thetaiotaomicron* (*B. theta*) or *E. coli* and a fecal transplant from a conventionally raised mouse. The *E. coli* inoculum was a 1:1 ratio of the EcN WT strain and the Δ*fdnG fdoG*. **(D)** EcN, *B. theta*, and *C. symbiosum* were subjected to the formate extraction protocol, treated with pure acetonitrile or mock-treated. Bacterial numbers at the end of the treatment were determined by plating on selective media. The experiment was performed in triplicate. ND, none detected. **(E)** Conventionally raised SPF and germ-free C57BL/6 mice were treated with 2 % DSS or water for 7 days. Representative images of hematoxylin and eosin-stained colonic sections from animals shown in Fig. 6C-D . Scale bar, 300 μm. **(F)** DSS-treated (3 %) SPF C57BL/6 mice were inoculated four days after the start of DSS treatment with the indicated EcN strains. NR, *narG napA narZ* mutant. Five days after inoculation, the competitive index in the cecum content was determined (animals shown in Fig. 7A). Bars represent geometric means ± standard error. The number of mice per group (N) is indicated in each panel. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not statistically significant.

SUPPLEMENTAL TABLES

Supplemental Table 1: Strains used in this study (related to Experimental Procedures)

Strain	Genotype	Reference
B. thetaiotaomicron		
ATCC 29148 (VPI 5482)	Δ*tdk*	(Koropatkin et al., 2008)
C. symbiosum		
ATCC14940		ATCC
E. cloacae		
CG36	Mouse commensal *E. cloacae* isolate; wild-type strain	This study
MW362	CG36 *moaA*::pMW2	This study
E. coli		
BW25113	K-12 parent strain; λ^- F^- *lacI*^{q} *rrnBT14* Δ*lacZWJ16 hsdR514* Δ*araBADAH33* Δ*rhaBADLD78*	(Datsenko and Wanner, 2000)
DH5α λ*pir*	F^- *endA1 hsdR17* (r^-m^+) *supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*)*U189* φ*80lacZ*Δ*M15* λ*pir*	(Pal et al., 2005)
ECK1468	BW25113 Δ*fdnG*767::Kan^r	(Baba et al., 2006)
ECK3887	BW25113 Δ*fdoG*757::Kan^r	(Baba et al., 2006)
EL200	SL1 Δ*fdnG*	This study
EL212	SL1 Δ*fdnG* Δ*fdoG*	This study
HS	Wild-type strain	(Levine et al., 1978)
LB21	NRG 857C Δ*fdnG*	This study
LB25	NRG 857C Δ*fdnG* Δ*fdoG*	This study
LB33	NRG 857C Δ*lacZ*	This study
MW153	EcN Δ*cyoABCD*	This study
Nissle 1917 (EcN)	Wild-type strain (O6:K5:H1)	(Grozdanov et al., 2004)
NRG 857C	Adherent invasive *E. coli* isolate (O83:H1) Carb^r Cm^r Tet^r	(Eaves-Pyles et al., 2008)
S17-1 λ*pir*	*zxx::*RP4 2-(Tet^r::Mu) (Kan^r::Tn7) λ*pir*	(Simon, 1983)
SL1	Mouse commensal *E. coli* isolate; wild-type strain	This study
SW930	EcN Δ*narG* Δ*napA* Δ*narZ*	(Winter et al., 2013)
SW1029	EcN Δ*moaA*	(Winter et al., 2013)
SW1075	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ*	This study
SW1099	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF*	This study
SW1104	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY*	This study
SW1123	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA*	This study
SW1124	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA*	This study

SW1130	EcN Δ*fdnG*	This study
SW1227	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* *nrfA*::pSW276	This study
SW1228	EcN *fdoG*::pSW278	This study
SW1229	EcN Δ*fdnG fdoG*::pSW278	This study
SW1351	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* Δ*fdnG*	This study
SW1355	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*fdnG*	This study
SW1358	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* Δ*fdnG fdoG*::pSW278	This study
SW1359	EcN Δ*narG* Δ*napA* Δ*narZ* Δ*fdnG fdoG*::pSW278	This study
SW1362	EcN Δ*cydA*	This study
SW1388	EcN Δ*cydA* Δ*fdnG*	This study
SW1390	EcN Δ*cydA* Δ*fdnG fdoG*::pSW278	This study
TOP10	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ*80lacZΔM15 lacX74 recA1 araD139* Δ(*ara - leu*)7697 *galU galK rpsL endA1 nupG*	ThermoFisher Scientific
WZ245	SL1 Δ*moaA*	This study
K. oxytoca		
CG57	Mouse commensal *K. oxytoca* isolate; wild-type strain, $Carb^r$	This study
MW363	CG57 *moaA*::pMW3	This study

Supplemental Table 2: Plasmids used in this study (related to Experimental Procedures)

Designation	Relevant characteristics	Reference
pCR2.1	*ori*(f1) Kan^r Amp^r *ori*(pUC) *lacZα*	ThermoFisher Scientific
pEL3	Upstream and downstream regions of the EcN *fdoG* gene in pGP706	This study
pEL5	Upstream and downstream regions of the HS *fdnG* gene in pGP706	This study
pGP704	*ori*(R6K) *mobRP4* Carb^r	(Miller and Mekalanos, 1988)
pGP705	*ori*(R6K) *mobRP4* Kan^r	This study
pGP706	*ori*(R6K) *mobRP4 sacRB* Kan^r	This study
pLB1	Upstream and downstream regions of the NRG857c *fdoG* gene in pGP706	This study
pLB2	Upstream and downstream regions of the NRG857c *fdnG* gene in pGP706	This study
pLB5	Upstream and downstream regions of the NRG857c *lacZ* gene in pGP706	This study
pMW2	*E. cloacae* CG36 *moaA* gene fragment in pGP705	This study
pMW3	*K. oxytoca* CG57 *moaA* gene fragment in pGP705	This study
pMW4	Upstream and downstream regions of EcN *cyoABCD* in pRDH10	This study
pRDH10	*ori*(R6K) *mobRP4* Cm^r Tet^r *sacRB*	(Kingsley et al., 1999)
pSW172	*ori*(R101) *repA101ts* Carb^r	(Winter et al., 2013)
pSW253	Upstream and downstream regions of the EcN *dmsA* gene in pCR2.1	This study
pSW254	Upstream and downstream regions of the EcN *dmsA* gene in pRDH10	This study
pSW255	Upstream and downstream regions of the EcN *torA* gene in pCR2.1	This study
pSW256	Upstream and downstream regions of the EcN *torA* gene in pRDH10	This study
pSW257	Upstream and downstream regions of the EcN *torZ* gene in pCR2.1	This study
pSW258	Upstream and downstream regions of the EcN *torZ* gene in pRDH10	This study
pSW259	Upstream and downstream regions of the EcN *yedY* gene in pCR2.1	This study
pSW260	Upstream and downstream regions of the EcN *yedY* gene in pRDH10	This study
pSW261	Upstream and downstream regions of the EcN *ynfF* gene in pCR2.1	This study
pSW262	Upstream and downstream regions of the EcN *ynfF* gene in pRDH10	This study

pSW269	Upstream and downstream regions of the EcN *fdnG* gene in pCR2.1	This study
pSW270	Upstream and downstream regions of the EcN *fdnG* gene in pRDH10	This study
pSW275	EcN *nrfA* gene fragment in pCR2.1	This study
pSW276	EcN *nrfA* gene fragment in pGP704	This study
pSW277	EcN *fdoG* gene fragment in pCR2.1	This study
pSW278	EcN *fdoG* gene fragment in pGP704	This study
pSW295	Upstream and downstream regions of the EcN *cydA* gene in pRDH10	This study
pUC4-KSAC	*ori*(pMB1) $Carb^r$ Kan^r	Pharmacia (GE Healthcare)
pWSK129	*ori*(pSC101) Kan^r	(Wang and Kushner, 1991)
pWSK29	*ori*(pSC101) $Carb^r$	(Wang and Kushner, 1991)
pWZ5	Upstream and downstream regions of the NRG 857c *moaA* gene in pGP706	This study

Supplemental Table 3: Primers for mutagenesis (related to Experimental Procedures)

Purpose/ target	**Sequence** (sequences homologous to suicide plasmids are underlined, restriction endonuclease cleavage sites are printed in bold, sequences homologous to genes of interest or flanking regions are printed in italics)
fdoG fragment from EcN	5'-**GCATGC***AGGCTTCGGTGTATTCGC*-3'
	5'-**GAATTC***CGATGACCAACCACTGGG*-3'
moaA fragment from *E. cloacae*	5'-CTAGAGGTACCGCATGC*AAGCCCGCATCACGCCAG*-3'
	5'-AGCTCGATATCGCATGC*ACGGCTACAAACCGGGCAG*-3'
moaA fragment from *K. oxytoca*	5'-CTAGAGGTACCGCATGC*TGACGTTAATCGCCGTCAG*-3'
	5'-AGCTCGATATCGCATGC*TATGCAACTTTCGTTGCAC*-3'
nrfA fragment from EcN	5'-**GTCGAC***CAACACCTGTAACTGTGG*-3'
	5'-CG**GAATTC***TCTTCGCCGTCTTTCTGG*-3'
Amplification of *sacRB*	5'-TCAACCGTGGCTCCA*TACCCATCGGCATTTTCTTTT*-3'
	5'-ATCCGTCGACCTGCAGATCT*AACCCATCACATATACCTGC*-3'
Deletion of *cyoABC* in EcN	5'-GCCATCTCCTTGCATG-C*TAATAAGTCATTTTCCGCTACTC*-3'
	5'-*CTTAGTGCTTAACGACCTCAATTCCAC*-3'
	5'-*GGTCGTTAAGCACTAAGAGCGGCGGTTATG*-3'
	5'-CAAGGAATGGTGCATG*CACAGCAGCCAGCAGCGT*-3'
Deletion of *cydA* in EcN	5'-GCCATCTCCTTGCATG*CTCTTGATTTTCAATCTTTGAGC*-3'
	5'-*TTAGCGTGGCTCATCGCATGAAGACTC*-3'
	5'-*CGATGAGCCACGCTAAGACAGGAGTCG*-3'
	5'-CAAGGAATGGTGCATGC*ACCCATACGCCAGCCAGT*-3'
Deletion of *dmsA* in EcN	5'-**GCATGC***GCTGGAAGAGATGACTGG*-3'
	5'-GAG**TCTAGA***GCTTGCTCACGATTG*-3'
	5'-GCG**TCTAGA***TGTTCAGGTTGAAAAGG*-3'
	5'-**GCATGC***TACTGAGTGCTGAAGCC*-3'
Deletion of *fdnG* in EcN	5'-**GGATCC***CTGAGTAACACGGCTGC*-3'
	5'-CG**TCTAGA***TCAGGGGTATTACTGCG*-3'
	5'-CC**TCTAGA***ATCGAGAAGGCGTAAGG*-3'
	5'-**GTCGAC***AATCACCACGGTCCAGT*-3'
Deletion of *fdnG* in NRG 857C	5'-CTAGAGGTACCGCATGC*AAGCAATGGACGTCAGTC*-3'
	5'-*CATCTGTTCGTTGGAGCAAGACTTGCTAC*-3'
	5'-*GCTCCAACGAACAGATGGCTATGGAAAC*-3'
	5'-AGCTCGATATCGCATGC*TAGACGCCAGCATGTTCG*-3'
Deletion of *fdnG* in SL1	5'-CTAGAGGTACCGCATGC*AGCCAGTTGGTTTTCTG*-3'
	5'-*CCGGTTGGGTATTACTGCGCGAGGAAAC*-3'
	5'-*CAGTAATACCCAACCGGGCCGTTTATC*-3'
	5'-AGCTCGATATCGCATGC*TGTTGACTAAGAACGCTTTATA TTCC*-3'
Deletion of *fdoG* in NRG 857C	5'-CTAGAGGTACCGCATGC*GTCAGCATGGTGCAGCAC*-3'
	5'-*GCAAGTCTTGGAAAAGGTGTAACGGAGAC*-3'
	5'-*CCTTTTCCAAGACTTGCTACCGTTGGTCC*-3'
	5'-AGCTCGATATCGCATGC*TCCCACTCCCTGCACACG*-3'

Deletion of *fdoG* in SL1	5'-CTAGAGGTACCGCATGC*GCGTACATTTGTAGACGC*-3'
	5'-CTCCAACATTGGAAAAGGTGTAACGGAG-3'
	5'-CCTTTTCCAATGTTGGAGCAAGACTTGCTACCGTTG-3'
	5'-AGCTCGATATCGCATGC*GGCAGGCACCACGGCAGC*-3'
Deletion of *lacZ* in NRG857c	5'-CTAGAGGTACCGCATG*CCACAGTTTTGGCTGTCTG*-3'
	5'-*TGTGTCCGGCCCGTATTTCGCGTAAG*-3'
	5'-*ATACGGGCCGGACACATAGAGTGTAAAG*-3'
	5'-AGCTCGATATCGCATG*CCAGACTCCCATCAACAG*-3'
Deletion of *moaA* in SL1	5'-CTAGAGGTACCGCATG*AAACGAGAACCCAAAGAC*-3'
	5'-*TTTGACGTTGTACACCTTTCCAGATACG*-3'
	5'-*AGGTGTACAACGTCAAAAGGAGAGATCAGATGAGTC*-3'
	5'-AGCTCGATATCGCATG*CGCGTACGGGCATCCAGC*-3'
Deletion of *torA* in EcN	5'-**GCATGC***GGAGAAAACCACCGTTGC*-3'
	5'-GCC**TCTAGA***CATCCTTCACCGTCG*-3'
	5'-GAC**TCTAGA***CAGAAGTATCCGTTGC*-3'
	5'-**GCATGC***AATCTCTTGCTCGTCC*-3'
Deletion of *torZ* in EcN	5'-**GCATGC***CAGTGACGATAAGTTC*-3'
	5'-GGG**TCTAGA***ATCAACGAGGATCACTG*-3'
	5'-ATA**TCTAGA***GACGTGAATAGCGCCC*-3'
	5'-**GCATGC***GGCGTGCTTTACCATCG*-3'
Deletion of *yedY* in EcN	5'-**GCATGC***CTGGACGCTTTTTCG*-3'
	5'-TAT**TCTAGA***TGTCGTTAGCCAGCAAG*-3'
	5'-CAA**TCTAGA***GCATCGCTGTATCGTG*-3'
	5'-**GCATGC***GAAACATAACCTGATG*-3'
Deletion of *ynfF* in EcN	5'-**GCATGC***AATGGGTATCTATAAGCG*-3'
	5'-CGC**TCTAGA***CACTTATTGCTTTTC*-3'
	5'-ATA**TCTAGA***GGAGTAGCCCATGACC*-3'
	5'-**GCATGC***ACGCCCATCAACAGCC*-3'
Generation of pGP705	5'-GATCCTTTTTGTCCGGTGTT-3'
	5'-AGATCTGCAGGTCGACGG-3'
	5'-CCGGACAAAAAGGATC*GAGCTCCTGCAGGGGGGG*-3'
	5'-GTCGACCTGCAGATCT*GGAGCCACGGTTGATGAGAGC*-3'
Generation of pGP706	5'-TCAACCGTGGCTCCA*TACCCATCGGCATTTTCTTTTG*-3'
	5'-ATCCGTCGACCTGC**AGATCT***AACCCATCACATATACCTGC*-3'

Supplemental Table 4: Primers used for RT-qPCR (related to Experimental Procedures)

Purpose/ target	**Sequence**	**Reference**
Gapdh (*mus musculus*)	5'-TGTAGACCATGTAGTTGAGGTCA-3'	(Overbergh et al., 2003)
	5'-AGGTCGGTGTGAACGGATTTG-3'	
Tnfa (*mus musculus*)	5'-AGCCAGGAGGGAGAACAGAAAC-3'	This study
	5'-CCAGTGAGTGAAAGGGACAGAACC-3'	
Nos2 (*mus musculus*)	5'-TTGGGTCTTGTTCACTCCACGG-3'	(Godinez et al., 2008)
	5'-CCTCTTTCAGGTCACTTTGGTAGG-3'	
Mip2 or Cxcl2 (*mus musculus*)	5'-AGTGAACTGCGCTGTCAATGC-3'	(Hu et al., 2004)
	5'-AGGCAAACTTTTTGACCGCC-3'	
Ifng (*mus musculus*)	5'-TCAAGTGGCATAGATGTGGAAGAA-3'	(Overbergh et al., 2003)
	5'-TGGCTCTGCAGGATTTTCATG-3'	
Cxcl1 (*mus musculus*)	5'-TGCACCCAAACCGAAGTCAT-3'	(Godinez et al., 2008)
	5'-TTGTCAGAAGCCAGCGTTCAC-3'	

Supplemental Table 5: Histopathology scoring (related to Experimental Procedures)

Score	Exudate	Epithelial Damage	Infiltration by PMNs*	Necrosis	Submucosal Edema
0	No changes	No changes	No changes (0-5)	No changes	No changes
1	Slight accumulation	Desquamation	6-20	Slight	Detectable (<10%)
2	Mild accumulation	Mild erosion	21-60	Mild	Mild (10%-20%)
3	Moderate accumulation	Marked erosion	61-100	Moderate	Moderate (20%-40%)
4	Marked accumulation	Ulceration	>100	Marked	Marked (>40%)
* Number of cells per high power field (400x)					

(the aggregate score in the figures refers to sum of individual score for each mouse)

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Bacterial strains. All *E. coli, K. oxytoca, and E. cloacae* strains were grown in LB broth (10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride) or on LB plates (LB broth, 15 g/L agar) at 37 ºC. When appropriate, antibiotics were added at the following concentrations: 100 µg/mL carbenicillin (Carb), 100 µg/mL kanamycin (Kan), and 30 µg/mL chloramphenicol (Cm). *B. thetaiotaomicron* was grown anaerobically (90 % N_2, 5 % CO_2, 5 % H_2; Sheldon Manufacturing) in tryptone-yeast extract-glucose (TYG) media (1 % tryptone, 0.5 % yeast extract, 0.2 % glucose, 0.05 % cysteine, 0.1 M potassium phosphate buffer pH 7.2, 1 µg/mL vitamin K solution, 20 µg/mL magnesium sulfate heptahydrate, 400 µg/mL sodium bicarbonate, 80 µg/mL sodium chloride, 8 µg/mL calcium chloride, 0.4 µg/mL iron sulfate heptahydrate, 1 µg/mL resazurin, 40 mM histidine pH 8, 240 µg/mL hematin) for 24 h or on blood plates (37 g/L brain heart infusion, 15 g/L agar, 5 % [v/v] defibrinated blood) containing 50 µg/mL gentamycin (Gent) for 2 days at 37 ºC. *C. symbiosum* was routinely cultured in pre-reduced chopped meat broth (BD) in Hungate tubes. Strains used are listed in Supplemental Table 1.

Isolation and characterization of mouse commensal Enterobacteriaceae isolates. All strains were isolated on MacConkey agar (17 g/L pancreatic digest of gelatin, 3 g/L peptones (meat and casein), 10 g/L lactose, 1.5 g/L bile salts no. 3, 5 g/L sodium chloride, 13.5 g/L agar, 0.03 g/L neutral red, 0.001 g/L crystal violet). *E. cloacae* CG36 was isolated from the intestinal tract of conventional Swiss Webster mice (Taconic Biosciences). The species of this isolate was confirmed by biochemical characterization (Enteropluri, Liofilchem). *K. oxytoca* CG57 was isolated from the intestinal tract of conventional CBA mice (Jackson Labs). Its species was confirmed through biochemical characterization (Enteropluri, BD). *E. coli* SL1 was isolated from the fecal contents of conventional C57BL/6 mice (Charles River) and confirmed through biochemical characterization (Enteropluri, BD).

Construction of plasmids. Plasmids were constructed with standard molecular cloning techniques or Gibson Assembly Cloning Kit (New England Biolabs) (J. Sambrook, 1989). Plasmids and primers used for DNA fragment amplification by PCR are listed in Supplemental Tables.

DNA regions upstream and downstream of *dmsA*, *torA*, *torZ*, *yedY*, *ynfF*, and *fdnG*, respectively, were PCR amplified from *E. coli* Nissle 1917 (EcN), digested with XbaI and ligated using T4 DNA ligase. Ligated DNA fragments were used as templates for PCR. The linked upstream and downstream regions were cloned into pCR2.1 using the TOPO TA cloning kit (ThermoFisher Scientific), generating pSW253, pSW255, pSW257, pSW259, pSW261, and pSW269, respectively. Plasmids inserts were sequenced prior to subcloning. To construct pSW254, pSW256, pSW258, pSW260, pSW262, and pSW270, the linked upstream and downstream regions of *dmsA*, *torA*, *torZ*, *yedY*, *ynfF*, and *fdnG*, respectively, were cloned into pRDH10 using SphI (pSW254, pSW256, pSW258, pSW260, pSW262) or SalI/BamHI (pSW270) restriction enzyme sites. pMW4 was generated by Gibson cloning of SphI-cut pRDH10 and PCR amplified regions upstream and downstream of *cyoABC* in EcN. To construct pSW275 and pSW277, an internal fragment of the *nrfA* and *fdoG*, respectively, was PCR amplified from EcN. The PCR product was cloned into pCR2.1 using the TOPO TA cloning kit (ThermoFisher Scientific) and sequenced prior to subcloning. pSW275 and pSW277 were digested with EcoRI and SphI and the internal fragments cloned into pGP704 using EcoRI/SphI restriction enzyme sites to give rise to pSW276 and pSW278, respectively. To construct pGP705, the Kan^r cassette of pUC4-KSAC and the *ori*(R6K)/*mobRP4* region of pGP704 were separately amplified by PCR and ligated in a Gibson cloning reaction (New England Biolabs). The *sacRB* genes of pRDH10 were PCR amplified and inserted into the BglII site of pGP705 by Gibson assembly to generate pGP706. To construct pMW2 and pMW3, internal fragments of the *moaA* gene in *E. cloacae* CG36 and *K. oxytoca* CG57, respectively, were PCR amplified and cloned

into SphI-digested pGP705, using Gibson cloning. Sequencing was used to verify inserts. To generate pLB1, pLB2, pLB5, and pWZ5, DNA regions upstream and downstream of *fdoG*, *fdnG*, *lacZ*, and *moaA*, respectively, were PCR amplified from NRG 857C. For pEL3 and pEL5, regions upstream and downstream of *fdoG* in EcN and *fdnG* in HS, respectively, were amplified. PCR products were inserted into the SphI site of pGP706 using the Gibson Assembly Cloning Kit (New England Biolabs). pSW295 was made by Gibson assembly of SphI-digested pRDH10 and upstream and downstream flanking regions of EcN *cydA*. Plasmid inserts were verified by sequencing.

Construction of mutants by allelic exchange. Suicide plasmids were propagated in DH5α λ*pir* and introduced into EcN (pSW172), NRG 857C, SL1 (pSW172), *E. cloacae* CG36 (pWSK29) and *K. oxytoca* CG57 via bacterial conjugation with the donor strain S17-1 λ*pir*. All conjugation experiments with EcN and SL1 were carried out at 30 ºC to enable stable replication of the heat sensitive plasmid pSW172. Exconjugates in which the suicide plasmid had integrated into the chromosome by single crossover were selected for on LB plates containing the appropriate antibiotics at the appropriate temperature. Integration of the plasmids in the chromosome was confirmed by PCR when appropriate. Then, second crossover events were selected for on sucrose plates (5 % sucrose, 15 g/L agar, 8 g/L nutrient broth base). This second event leads to an unmarked deletion, which was confirmed by PCR. If appropriate, pSW172 was cured by growing the bacteria overnight at 37 ºC.

The EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* mutant (SW1124) was generated in SW930 (Δ*narG* Δ*napA* Δ*narZ)* by subsequent introduction of the suicide plasmids pSW258, pSW262, pSW260, pSW254, pSW256 via conjugation followed by sucrose selection, generating SW1075 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ*), SW 1099 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF*), SW1104 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY*), and SW1123 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA*) in the process.

Exconjugates were recovered on LB plates containing Carb (100 µg/mL) and Cm (30 µg/mL) at 30 ºC.

pSW295 and pMW4 were introduced via conjugation into EcN to generate EcN Δ*cydA* (SW1362) and Δ*cyoABC* (MW153), respectively, by selection of Carb^r and Cm^r exconjugates which were then subject to sucrose selection. To create the EcN Δ*fdnG* mutant (SW1130), the suicide plasmid pSW270 was introduced via conjugation. Then, Carb^r and Cm^r exconjugates were used in sucrose selection. SW1130 was then used to generate SW1229 (Δ*fdnG fdoG*::pSW278) by introduction of pSW278 and selection of Carb^r and Kan^r exconjugates. Similarly, SW1228 (*fdoG*::pSW278), SW1351 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* Δ*fdnG*), SW1358 (Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA* Δ*fdnG fdoG*::pSW278), SW1355 (Δ*narG* Δ*napA* Δ*narZ* Δ*fdnG*), SW1359 (Δ*narG* Δ*napA* Δ*narZ* Δ*fdnG fdoG*::pSW278), SW1388 (Δ*cydA* Δ*fdnG*), and SW1390 (Δ*cydA* Δ*fdnG fdoG*::pSW278) were constructed. The plasmid pSW276 was introduced into SW1124 and the EcN Δ*narG* Δ*napA* Δ*narZ* Δ*torZ* Δ*ynfF* Δ*yedY* Δ*dmsA* Δ*torA nrfA*::pSW276 mutant (SW1227) was selected for on LB plates containing Carb and Kan.

The NRG 857C Δ*fdnG* Δ*fdoG* mutant (LB25) was generated by subsequent introduction of pLB2 and pLB1 by conjugation, selection on LB containing Kan and Carb, and sucrose selection, generating LB21 (Δ*fdnG*) in the process. The NRG 857C Δ*lacZ* mutant (LB33) was generated by introduction of pLB5 via conjugation, selection with Kan and Carb, followed by sucrose selection. LB33 was confirmed as growing white on LB plates containing 80 µg/L of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal).

For SL1, plasmids with insert sequences from other *E. coli* strains were used due to sequence similarity. SL1 Δ*moaA*, Δ*fdnG*, and Δ*fdnG* Δ*fdoG* (WZ245, EL200, and EL212) were constructed by introducing pWZ5, pEL5, and pEL3 via conjugation, selecting for Kan^r and Carb^r exconjugates, and performing sucrose selection.

The *moaA*::Kan^r mutants in *E. cloacae* CG36 (MW362) and *K. oxytoca* CG57 (MW363) were generated via conjugation with S17-1 λ*pir* strains carrying pMW2 and pMW3, respectively. Exconjugates were selected for on LB plates containing Carb and Kan.

DSS-induced colitis model in conventional mice. Male and female 6-12 week old C57BL/6 and Swiss Webster wild-type mice were used. To induce colitis, the drinking water was replaced with a filter-sterilized solution of 2 % [w/v] or 3 % [w/v] dextran sulfate sodium (DSS; Alfa Aesar) in water (Winter et al., 2013). DSS-treated mice were switched to regular drinking water 1 day prior to the end of the experiment. At the indicated time points, mice received 1 x 10^9 CFU or 1 x 10^5 CFU of the *E. coli*, *K. oxytoca*, or *E. cloacae* indicator strains in LB broth (by intragastric gavage), as indicated. In the competition experiments, mice received 5 x 10^8 CFU or 5 x 10^4 CFU of each *E. coli*, *K. oxytoca*, or *E. cloacae* strain (as indicated). Animals were euthanized 7 or 9 days after the start of DSS treatment (as indicated). Colonic and cecal tissue for histopathology analysis was collected. Colonic tissue for quantification of mRNA levels was flash frozen and stored at -80 ºC. Cecum and colon content was collected in sterile phosphate buffered saline (PBS; pH = 7.4) for bacterial counts or formate measurements. The bacterial loads of the respective strains were determined by plating serial 10-fold dilutions on LB plates containing the appropriate antibiotics. To facilitate recovery from biological samples and to compare strains, the *E. cloacae* CG36 and *E. coli* EcN, K-12 (BW25113), and SL1 strains were marked with the low-copy number plasmids pWSK29 (Carb^r) and pWSK129 (Kan^r). These plasmids have been previously reported to be stably replicated during the course of the experiment and to have no effect on the bacterial load of EcN (Winter et al., 2013). The *K. oxytoca* CG57 and *E. coli* NRG 857c strains are naturally resistant to Carb, and thus did not need to be marked with plasmids. For the NRG 857C competition experiment, LB plates were additionally

supplemented with 80 µg/L of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to distinguish between the *lacZ*-encoding and Δ*lacZ* strains.

DSS-induced colitis model in gnotobiotic mice. Germ-free C57BL/6 and Swiss Webster mice received filter-sterilized 2 % DSS and 3 % DSS solutions, respectively, or filter-sterilized water (mock control). The concentration of DSS in germ-free C57BL/6 mice was reduced to 2 % and the time course reduced to 7 days since germ-free animals that received 3 % DSS developed severe disease symptoms and had to be euthanized. DSS-treated Swiss Webster mice were switched to filter-sterilized water 1 day prior to the end of the experiment (8 days after the start of DSS treatment). Three days prior to the start of DSS treatment, germ-free Swiss Webster mice were intragastrically inoculated with one of the following: *E. coli* indicator strains alone (5×10^8 CFU of the EcN wild-type strain and 5×10^8 CFU of the Δ*fdnG fdoG* mutant), the *E. coli* indicator strains and *B. thetaiotaomicron* (3×10^9 CFU), or the *E. coli* indicator strains and a mouse fecal transplant from a C57BL/6 mouse with a conventional microbiota. To prepare the fecal transplant, the large and small intestines were collected from a healthy male C57BL/6 donor in anaerobic, sterile tissue culture media (RPMI-1640 Medium; Sigma-Aldrich). Under anaerobic conditions, the colon and cecum content was resuspended in filter-sterilized PBS and passed through a cell strainer (70 µm Nylon; Corning) to remove large particles. At the end of the experiments, sample collection proceeded as described above. In addition, colon and cecum content from mice treated with *B. thetaiotaomicron* was plated on blood agar supplemented with gentamycin, to confirm successful colonization with *B. thetaiotaomicron* (data not shown).

Piroxicam-accelerated colitis model in conventional *Il10* knockout mice. Conventional *Il10* knockout mice on C57BL/6 (males only) and BALB/c (males and females) backgrounds were given Piroxicam chow (50 ppm for all groups, except 100 ppm for the *Il10* knockout C57BL/6 mice treated with SL1; Teklad custom research diets,

Envigo) instead of regular mouse chow at the start of the experiment. Piroxicam chow was changed daily. Two days after start of piroxicam treatment, mice received 1 x 10^9 CFU of the *E. coli* indicator strains in LB broth (by intragastric gavage). *Il10* KO C57BL/6 mice were colonized for 7 days, whereas *Il10* KO BALB/c mice were colonized for 14 days. At the end of the experiments, sample collection proceeded as outlined previously. Mice which were not colonized were excluded from analysis.

T cell transfer model of colitis in conventional *Rag1* knockout mice. Spleens of SPF housed C57BL/6 mice were isolated, placed in ice cold RPMI + 10 % FBS, and homogenized using frosted slides. Cells were resuspended and passed through a 70 μm cell strainer to remove tissue clumps. Naïve T cells were then isolated using MojoSort™ Mouse CD4 T Cell Isolation Kit (catalog no. 480006) according to the manufacturer`s recommendations. Isolated T cells were blocked (Rat anti-mouse CD16/CD32, clone 2.4G2, BD Pharmingen, catalog no. 553142) and stained for CD4 and CD45RB (Anti-mouse CD4-FITC, clone RM4-5, eBioscience, catalog no. 11-0042-82; Anti-mouse CD45 RB-APC, clone C363-16A, eBioscience, catalog no. 17-0455-81) at a 1:250 and 1:200 ratio, respectively. CD4(+) and CD45RB(high) T cells (Naïve T cells) were sorted and 3.6 x 10^5 cells were intraperitoneally injected into each *Rag1* -/- mouse. After T cell transfer, mouse health and body weights were monitored. Once weights began to stabilize and decline, fecal pellets were collected to test for occult blood (Hemoccult, Beckman Coulter). Feces were collected twice: on day 0 and day 2. Cages in which 50 % or more of the mice tested positive for occult blood on both occasions were inoculated on day 6, with each mouse receiving 5 x 10^8 CFU of each *E. coli* indicator strain (SL1 wild-type and SL1 Δ*fdnG* Δ*fdoG*). On day 15 (9 days of colonization), mice were sacrificed and samples collected as outlined above.

Metagenomics. Genomic DNA from cecal content was isolated using the PowerFecal DNA Isolation kit (MoBio), according to the recommendations of the

manufacturer. DNA was incubated with excess amounts of KCl on ice to precipitate residual DSS. The samples were then cleared by centrifugation and the resulting supernatant was subsequently subjected to ethanol precipitation to recover the DNA. The DNA concentration was measured using the Qubit dsDNA HS kit (Life Technologies) and the DNA was sonicated into 100-900 bp fragments using an S2 focused ultrasonicator (Covaris). The sheared DNA was analyzed on a Bioanalyzer 2100 (Agilent Technologies) to determine the quality of the DNA fragments for library preparation. Illumia sequencing libraries were generated using the KAPA high throughput library preparation kit (KAPA Biosystems) with six PCR cycles. Six bp indexed Illumia Truseq adapters were incorporated to facilitate demultiplexing of the reads. DNA libraries were size selected to a target range of 300-1000 bp using AMPure XP beads (Beckman Coulter) and library quality and concentration was determined using the Bioanalyzer 2100. Libraries were sequenced by Illumia NextSeq with a read length of 150 bp (paired end). Approximately 10 million reads were generated per sample.

To remove potential contaminating reads, the raw reads were mapped against the internal Illuimna control phiX174 (J02482.1) and the mouse mm10 and human hg38 genomes (UCSC Genome Browser, https://genome.ucsc.edu/) using the BBsplit tool, a component of the BBMap short read aligner tool set (Version 34.86, https://sourceforge.net/projects/bbmap). The following parameters were used: match=long, fastareadlen=500, minapproxhits=2, minratio=0.9, maxindel=20, trim=both, untrim=true, ambig2=split. All unmapped reads were written to separate files and further processed to remove index adapters using BBduk which is part of the BBMap software suite with the following parameters: ktrim=true, k=20, mink=4, minlength=20 and qtrim=false. These decontaminated and adapter trimmed read sets were used for all downstream analysis.

To process the sequencing reads for use in MEtaGenome ANalyzer version 5 (MEGAN5) (Huson et al., 2007), paired end reads were interleaved into single fasta files using the BBMap reformat tool. A blastX database was created in DIAMOND (Buchfink et al., 2015) using the non-redundant protein database downloaded from NCBI in October of 2015. Interleaved reads were aligned to the blastX database using DIAMOND and the output files were converted by DIAMOND to m8 files using the -view function and directly loaded into MEGAN5. Taxonomic comparisons were performed in MEGAN5 using the default parameters. Metabolic pathway analysis was performed for each sample by comparing the blastx results to the KEGG and SEED databases downloaded in February of 2015. Reads mapped to the SEED database were exported from Megan5 into BIOM tables, which were subjected to Analysis of similarity (ANOSIM) in Qiime (Caporaso et al., 2010) and Principle Component Analysis (PCA) using STAMP (Shavit et al., 2014). To map reads to bacterial metabolic genes, a total of 100 each of the Enterobacteriaceae formate dehydrogenase and terminal oxidase operons (*fdn*, *fdo*, *cyd*, and *cyo*) were downloaded from KEGG. Sequences were clustered to remove redundancy using cdhit-est (Fu et al., 2012; Li and Godzik, 2006) with a sequence identity threshold of 0.9. Paired end reads were mapped to these gene clusters using the BBmap tool from the BBMap short read aligner with the following settings: qtrim=lr, minid=0.90, ambig=random, covstats=true. Coverage statistics for each gene cluster were tallied from the percent unambiguous and ambiguous mapped reads and multiplied by the total number of reads used for mapping to determine the percent of reads that matched a particular gene set.

Quantification of mRNA levels in intestinal tissue. As described previously, the relative transcription levels of TNF-α, iNOS, Mip2 (Cxcl2), IFN-γ and KC (Cxcl1) (encoded by *Tnfa, Nos2*, *Cxcl2, Ifng*, and *Cxcl1* genes) were determined by RT-qPCR and normalized to *Gapdh* mRNA levels (Winter et al., 2009). Briefly, after colonic tissue

homogenization with a Mini-BeadBeater (BioSpec Products), RNA was extracted via the TRI reagent method (Molecular Research Center). To remove DSS contamination, mRNA was purified using the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). To synthesize cDNA from mRNA, TaqMan reverse transcription reagents (Life Technologies) were used. qPCR was performed with SYBR Green (Life Technologies) in an 11 µL reaction volume with 2 µL of cDNA as template. The primers listed in Supplemental Table 4 were added at a final concentration of 250 nM. Data was acquired in a QuantStudio 6 Flex Instrument (Life Technologies). For Mip2 and IFN-γ, results were analyzed using the comparative Ct method. For TNF-α, iNOS, and KC absolute counts of *Gapdh* (housekeeping gene), *Tnfa*, *Nos2*, and *Cxcl1* were determined based on standard curves, generated by serial dilutions of plasmids with the gene of interest cloned into the TOPO cloning vector (Invitrogen). For Figure S6C, one sample was excluded from analysis in the piroxicam-treated *Il10* KO BALB/c mice due to lack of amplification for all genes measured.

Histopathology. Cecal and colonic tissue was fixed with 10 % buffered formalin phosphate (Thermo Fischer), embedded in paraffin, and 5 µm sections of the tissue were stained with hematoxylin and eosin. The samples were blinded and lesions (submucosal edema, infiltration by neutrophils, epithelial damage, presence of exudate) scored as described in Supplemental Table 5. A combined score refers to the sum of scores for a particular tissue sample.

Measurement of formate concentrations in colon content by GC-MS. Colon content from mice was collected in PBS, weighed, and supplemented with deuterated sodium formate (Sigma-Aldrich) as an internal control. After 2 min of vortexing to disperse the content, bacteria and cells were removed by two cycles of centrifugation at 3,200 g for 15 min at 4 ºC. The supernatant was stored at -80 ºC. Sodium formate (Sigma-Aldrich) supplemented with deuterated sodium formate was used to make filter-

sterilized standard solutions in PBS, which were also stored at -80 ºC. External standards and biological samples were derivatized as follows: To protonate formate, 2 M hydrochloric acid was added in a 1:1 ratio to the samples. Liquid-liquid extraction was performed to extract formic acid into ethyl acetate (Sigma-Aldrich). Anhydrous sodium sulfate (Sigma-Aldrich) was added to remove any remaining water. The ethyl acetate extract of formic acid was incubated at 80 ºC for 1 h with the derivatization reagent *N*-*tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide with 1 % t-BDMCS (Cerilliant). Derivatized samples were transferred to autosampler vials for gas chromatography-mass spectrometry (GC-MS) analysis (Shimadzu, TQ8040). The injection temperature was 250 °C and the injection split ratio was set to 1:100 with an injection volume of 1 µL. The oven temperature started at 30 °C for 4 min, increasing to 230 °C at 10 °C per min and to 330 °C at 20 °C per min with a final hold at this temperature for 30 s. Flow rate of the helium carrier gas was kept constant at a linear velocity of 50 cm/s. The column used was a 30 m × 0.25 mm × 0.25 µm Rtx-5Sil MS (Shimadzu). The interface temperature was 300 °C. The electron impact ion source temperature was 200 °C, with 70 V ionization voltage and 150 µA current. For qualitative experiments, Q3 scans (range of 50-500 m/z, 1000 m/z per second) were performed. The retention time for formate and deuterated formate was 7.3 min (retention index of 842). For quantitative experiments, Q3 selected ion monitoring (single quadrupole mode) with an event time of 0.05 s was performed. The target and reference (qualifier) ions for formate were m/z = 103 and m/z = 75, respectively; target and reference ions for deuterated formate were m/z = 104 and m/z = 76.

Mucin broth growth assay. Mucin broth was prepared by dissolving hog mucin (0.5 % [w/v]; Sigma-Aldrich) in No-Carbon E medium supplemented with trace elements (Price-Carter et al., 2001; R. W. Davis, 1980). Sodium nitrate, DMSO and TMAO were added at a final concentration of 40 mM each. The final concentration of sodium formate was 20 mM. Two mL of mucin broth were inoculated with the indicated *E. coli* strains at

a final concentration of 1 x 10^3 CFU/mL and incubated microaerobically (standing culture), aerobically (shaking culture, 250 rpm) or anaerobically (90 % N_2, 5 % CO_2, 5 % H_2; Sheldon Manufacturing) for 18 h (*E. coli* Nissle 1917 experiments) or 16 h (NRG 857c experiments) at 37 ºC.

Growth curve. Growth curves were performed in filter sterilized M9 minimal medium (12.8 g/L sodium phosphate dibasic heptahydrate, 3 g/L potassium phosphate monobasic anhydrous, 0.5 g/L sodium chloride, 1 g/L ammonium chloride, 0.4 % [w/v] glucose, 2 mM magnesium sulfate, 0.1 mM calcium chloride). Overnight cultures of the indicated *E. coli* strains were diluted in M9 medium, at a final concentration of 1 x 10^8 CFU/mL. Bacterial cultures were then incubated under aerobic (250 rpm) or anaerobic (in M9 medium pre-incubated under anaerobic conditions; 90 % N_2, 5 % CO_2, 5 % H_2; Sheldon Manufacturing) growth conditions at 37 ºC. The optical density at 600 nm (OD_{600}) was measured every 30 min. The experiment was performed in triplicate for each strain.

Nitrate reductase assay. Twenty μL of LB broth supplemented with 40 mM sodium nitrate were used to dilute overnight cultures of the indicated strains at a 1:100 ratio. The diluted bacterial cultures were transferred into 384 well plates and incubated at 37 ºC microaerobically (without shaking) for 2 h before reading the OD_{600}. Forty μL of a 1:1 mix of sulfanilic hydrochloride and *N*-(1-Naphthyl) ethylenediamine dihydrochloride (Marshall's reagent) were then added to the cultures. After mixing, the cultures were incubated for 10 min. OD_{420} and OD_{540} were measured using a BioTek plate reader and the nitrate reductase activities were calculated using the following formula: NR Activity = $(OD_{540} - 0.72 \times OD_{420})/(0.06 \times 120 \times OD_{600})$.

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